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Transdermal delivery of anticholinergic bronchodilators

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Chapter 6

Evaluation of a novel diffusion cell for in vitro transdermal permeation: Effects of injection height, volume and temperature.

6.0. Summary

The objective of this study was to evaluate the performance of a new, compact, dynamic diffusion cell for in vitro transdermal permeation. These so-called Kelder-cells were developed as an automated alternative for the static Franz diffusion cells. The new cells were used in combination with the ASPEC-system (Automatic Sample Preparation with Extraction Columns) which was initially designed for the automation of solid-phase extractions. Three variables were tested to optimize the performance of the new cell system: Injection height into the inlet compartment, volume flowing through the receptor compartment and temperature. Experiments were performed using the tritium labelled anticholinergic [^3H]dexetimide permeating through an artificial membrane (Silastic®).

The injection height of the needle into the inlet compartment of the cell should be programmed at -34 mm to ensure complete air tightness and to force the buffer to flow through the cell. The volume of buffer flowing through the receptor compartment is important to maintain sink conditions: A volume of 117 μl was chosen to replace the total content of the cell (84 μl) every two minutes. The temperature was precisely controlled in a thermostatic cabinet to minimize variations in experimental conditions. For [^3H]dexetimide, an increase in temperature of 20°C reduced the lag time with a factor of about two, however the influence on the flux was negligible. The data of the Kelder-cells were comparable with static Franz diffusion cells, however Kelder-cells have the advantage of automatic sampling, continuous replacement of the receptor solution, and unattended operation over at least 24 hours.

6.1. Introduction

In vitro skin permeation of a drug can be studied using a static diffusion cell or a dynamic flow-through cell [1-3]. The main difference is that in the flow-through cell the receptor fluid flows below the skin, so that saturation of this fluid with the chemical of interest does not occur, as may be the case in the static system [4].

One of the most widely used static designs for studying in vitro permeation is the Franz diffusion cell [1, 2]. Permeation of chemicals is monitored by sampling the stirred receptor solution. This manual procedure is labour intensive, requires constant attention and sampling is often limited to the normal laboratory hours.

Recently we developed a new, compact, dynamic diffusion cell for in vitro transdermal permeation [5]. The main objective in designing these so-called Kelder-cells was to develop an automated alternative for the Franz diffusion cells. The Kelder-cells were used in combination with the ASPEC-system (Automatic Sample Preparation with Extraction Columns) which was initially designed for the automation of solid-phase extractions (SPE) [6]. The cells mimic the blood flow beneath the skin by replacement of the receptor solution every two minutes. The collection of the receptor solution is flexible and reproducible and the volume and flow rate through the receptor compartment of the cell can be varied.

In previous experiments we focused on the development of the design and experiments were not temperature controlled [5]. However, the temperature of an in vitro system should be controlled at a target temperature to minimize variation in experimental conditions. Therefore, the ASPEC-system was now placed in a temperature controlled cabinet to provide a uniform temperature of the entire system.

The purpose of the present study was to evaluate the performance of the new cell system by testing the following variables: Injection height into the inlet compartment, volume flowing through the receptor compartment and temperature. Experiments were performed using the tritium labelled anticholinergic [^3H]dextimide permeating through an artificial membrane (Silastic®) which was used as a model skin. The results are discussed and compared with those obtained using the static Franz diffusion cell.

6.2. Materials and Methods

6.2.1. Materials

[^3H]Dextimide hydrochloride (12.6 Ci/mmol) was obtained from Janssen Pharmaceutica N.V. (Beerse, Belgium). 1-Dodecylazacycloheptan-2-one (Azone®) was kindly supplied by Nelson Research (Irvine, CA, USA). Viton® O-rings were obtained from Eriks (Alkmaar, The Netherlands). All other chemicals and solvents were of analytical grade and obtained from Merck (Darmstadt, Germany). Polyethylene tubes (12 ml) were obtained from Greiner (Alphen a/d Rijn, The

Netherlands). Rialuma was used as scintillation liquid, obtained from Lumac (Olen, Belgium), in combination with mini-scintillation counting vials from Packard (Groningen, The Netherlands).

6.2.2. Preparation of solutions

Isotonic phosphate buffered saline pH 7.4 (PBS-buffer) was prepared by dissolving 8.00 g NaCl, 0.20 g KCl, 0.20 g KH_2PO_4 and 1.44 g $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ in 1 l distilled water.

The drug solution of [^3H]dexetimide was prepared by mixing 20 μl of an ethanolic stock solution of [^3H]dexetimide (3 MBq/ml) with 430 μl of ethanol / propylene glycol / PBS-buffer / Azone® 60:20:15:5 v/v [7].

6.2.3. Preparation of Silastic® membranes

Non-reinforced silicone membrane (Silastic®, polydimethylsiloxane, type 500-1, Laboratoire Perouse Implant, Bornel, France) of 0.125 mm thickness was extensively rinsed in hot distilled water (60°C) until all sodium bicarbonate (present on the surface to facilitate handling) was removed. This was followed by a thorough rinse in distilled water (20°C) for one hour [8].

6.2.4. Permeation experiments with Kelder-cells

Kelder-cell

The Kelder-cells (University Centre for Pharmacy, Groningen, The Netherlands) were made compatible with the ASPEC-system (Gilson Medical Electronics, Villiers le Bel, France) and the system was placed in a temperature controlled cabinet. Five racks were placed in the polypropylene tray and five blocks, each containing four cells, were placed in these racks. The Kelder-cell consists of an inlet compartment (A), a donor compartment (B) and a receptor compartment (C) (Figure 6.1). A circular piece of membrane (D) with a diameter of 12 mm was cut and placed into the receptor compartment which was filled with PBS-buffer. A Viton® O-ring (E) was used to position the membrane between the donor and receptor compartment. The cell is very compact with a contact area of 0.51 cm^2 and a receptor compartment volume of 77 μl [5]. After 30 min of equilibration of the membrane with the PBS-buffer, 75 μl of the drug solution was applied in the donor compartment by means of a pipet. The donor compartment was covered with parafilm to prevent evaporation of the solvent. The inlet compartment of the cell was sealed with a polypropylene cap, suitable for injection purposes (J). For each cell, 12 polyethylene tubes (12 ml) were positioned below the cell, to collect subsequent fractions of the receptor solution with permeated chemical.

The interval time between subsequent fractions, the dispensing volume, and the flow rate of the buffer through the cell can be varied.

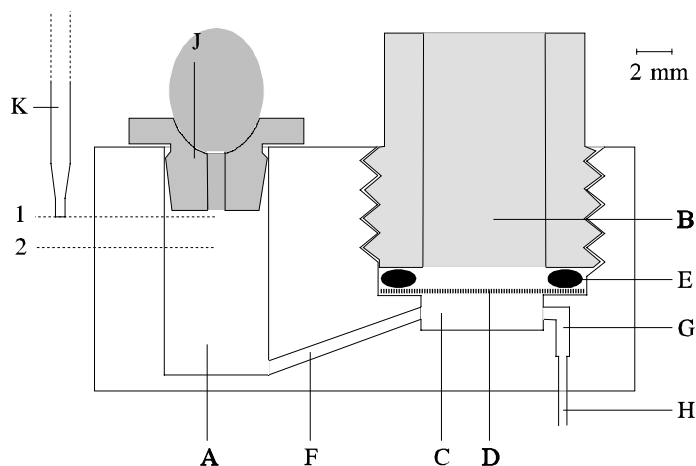


Figure 6.1. Cross-sections of the Kelder-cell.

A = inlet compartment; B = donor compartment; C = receptor compartment; D = membrane; E = O-ring; F = inlet channel; G = outlet channel; H = outlet tube; J = polypropylene cap; K = needle; 1 = injection height of -32 mm; 2 = injection height of -34 mm.

Permeation experiments

The ASPEC-system was programmed to run permeation experiments for 24 hours, which means that samples of two hours were collected in every polyethylene tube. To optimize the performance of the system, different variables were tested: Injection height of the needle into the inlet compartment, dispensing volume of receptor fluid flowing through the cell and temperature.

The injection height can be programmed to move the needle vertically up or down and this is important to ensure air tightness when the needle enters the polypropylene cap of the inlet compartment. To position the needle (Figure 6.1, K) at a lower level than height 0, which is defined as the position of the needle in the home position, a negative value should be programmed. The tested injection heights were -32 mm and -34 mm as shown in Figure 6.1 (temperature 22°C, collection volume 9 ml).

The tested volumes collected during two hours in a polyethylene tube were 4.5 ml, 7 ml and 9 ml, respectively (temperature 22°C, height -34 mm). This corresponds with 60 aliquots of 75 µl, 117 µl and 150 µl injected into the cells sequentially every two minutes. The temperature experiments were performed at 22°, 27°, 32°, 37° and 42°C (height -34 mm, collection volume 7 ml).

In all experiments, the flow rate through the injection needle was programmed at 50 µl/s and the interval time between subsequent rinsings of one cell was in the order of two minutes (discontinuous flow-through) [5].

6.2.5. Permeation experiments with Franz diffusion cells

The experiments were performed according to a previously described procedure [5]. The drug solution of [^3H]dextimide was prepared by mixing 10 μl of an ethanolic stock solution of [^3H]dextimide (3 MBq/ml) with 500 μl of ethanol / propylene glycol / PBS-buffer / Azone® 60:20:15:5 v/v and 200 μl of this dosing solution was applied in the donor compartment.

6.2.6. Analytical procedure

To determine the concentration of [^3H]dextimide in the receptor solution, 1 ml of the receptor solution sample was added to mini-scintillation vials and mixed with 3.5 ml Rialuma. The vials were counted for 40,000 counts or 5 min in a liquid scintillation counter (Minaxi, Packard, Groningen, The Netherlands), whatever came first .

6.2.7. Data analysis

Using Kelder-cells, the amount of permeated drug was calculated based on the measured concentrations in the receptor compartment, and the collected volume. Using Franz diffusion cells, the measured concentrations were corrected for the dilution during sampling. The results of the permeation experiments were plotted in graphs showing the percentage of permeated [^3H]dextimide versus time. Flux values were calculated by linear regression from the steady state portion of the permeation curve. The lag time was determined by extrapolation of the steady state portion of the curve to the intercept of the time axis.

6.3. Results and Discussion

6.3.1. Effect of injection height, volume and temperature

To optimize the performance of the Kelder-cells, different parameters were varied in the programming of the ASPEC-system. One important parameter is the injection height of the needle into the inlet compartment of the cell. The range in varying this height is narrow because if the needle moves too deep into the inlet compartment, it will be jammed. However, if the needle moves not deep enough, the buffer cannot be forced to flow through the cell. Figure 6.2 shows the mean cumulative percentage of [^3H]dextimide permeation, using two different injection heights, -32 mm and -34 mm. For the tested heights, no significant differences in permeation were found at any time interval (Student t-test, $p > 0.05$). In further experiments, we used the height of -34 mm to ensure complete air tightness of the cells.

Another important parameter is the volume of buffer pumped through the receptor compartment of the cell. In order to maintain sink conditions, the volume pumped

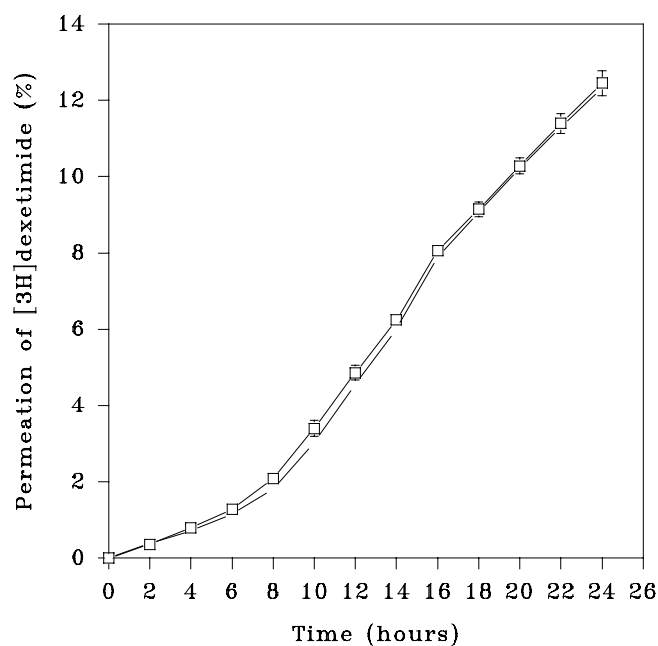


Figure 6.2. *Effect of injection height on the permeation of [^3H]dextimide through Silastic® membranes at 22°C.*
 □ = - 32 mm (n=3); ▼ = -34 mm (n=2); each point represents the mean and standard error of the mean.

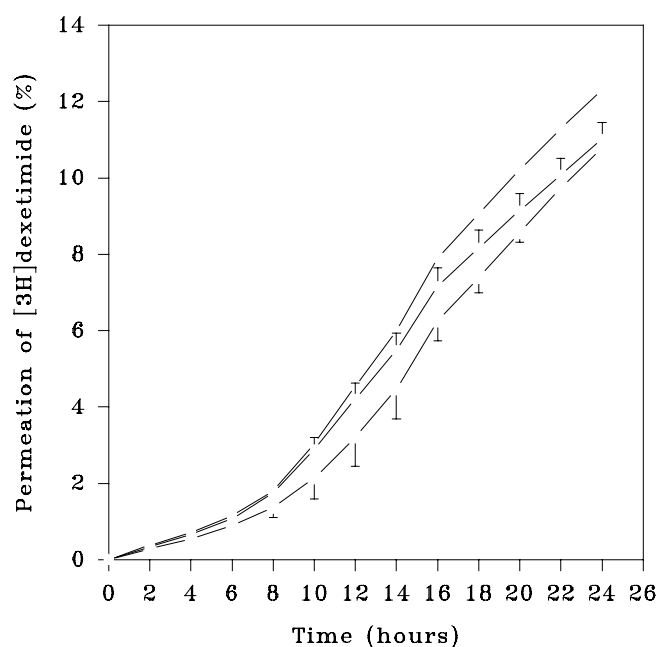


Figure 6.3. *Effect of collection volume on the permeation of [^3H]dextimide through Silastic® membranes at 22°C.*
 ▲ = 4.5 ml (n=2); ■ = 7 ml (n=4); ● = 9 ml (n=2) collected in two hours; each point represents the mean and standard error of the mean.

through the cell in a given time should be many times the volume of the receptor compartment [9-10]. In Figure 6.3, the permeation of [^3H]dextetamide is shown, using three different volumes of 4.5 ml, 7 ml and 9 ml, respectively, collected in two hours. The results indicate that with an increase in collection volume, the permeation of [^3H]dextetamide increases. However, comparison of the permeated amounts of [^3H]dextetamide showed no significant differences between the three volumes at any time interval (one-way ANOVA, $p > 0.05$). In further experiments, we collected 7 ml fractions in 2 hours, which corresponds with 60 injections of 117 μl aliquots. Using this volume, the total content of the cell (84 μl) is replaced by fresh buffer every two minutes.

Temperature control is also an important factor in permeation experiments. Many of the diffusion cells in use have a water jacket to control the temperature, however using this type of cells, only the receptor compartment is thermostated [1, 2]. We performed temperature experiments of [^3H]dextetamide in a thermostatic cabinet which controlled the temperature of the entire system precisely ($\pm 1^\circ\text{C}$). Figure 6.4 shows the mean cumulative percentages of [^3H]dextetamide permeation at five temperatures and for clarity reasons the corresponding standard errors of the mean (sem) are presented in Table 6.1. At 37°C , the standard errors of the mean are much higher compared to other temperatures, which may be explained by swelling of the Silastic® membrane [8].

With an increase in temperature, the lag time reduced from about 5 h at 22°C to 2.5 h at 42°C , but the influence on the flux was negligible. These results are not in line with the theoretical guideline which describes that a 10°C rise in temperature can produce a doubling in permeation although only limited examples are published which illustrated this influence [1, 8, 11]. In other experiments, changes in permeability coefficients appear to be small for temperatures up to 70°C indicating that the overall effect on in vitro skin permeation is small. Above 70°C , irreversible denaturation may occur and large increases in the permeability coefficient could be observed [12].

6.3.2. Comparison of Kelder-cell and Franz diffusion cell

The Kelder-cell and the static Franz diffusion cell were compared using the compound [^3H]dextetamide. The receptor compartments of the Franz diffusion cells were kept at 37°C through an external water jacket which may result in a membrane temperature of approximately 32°C [11]. Therefore, we compared these data with the experiments of the Kelder-cells performed at 32°C (Figure 6.5). Although in the beginning of the curves, the two cell types differed ($t = 4$ and 6 h, Student t -test, $p < 0.05$), no significant differences were found in the latter end of the curves (Student t -test, $p > 0.05$). Probably, the amount of [^3H]dextetamide permeated through the membrane was too small to require continuous replacement of the receptor solution. The variability in the data expressed as the coefficient of variation was also comparable for the two cell types (Table 6.2).

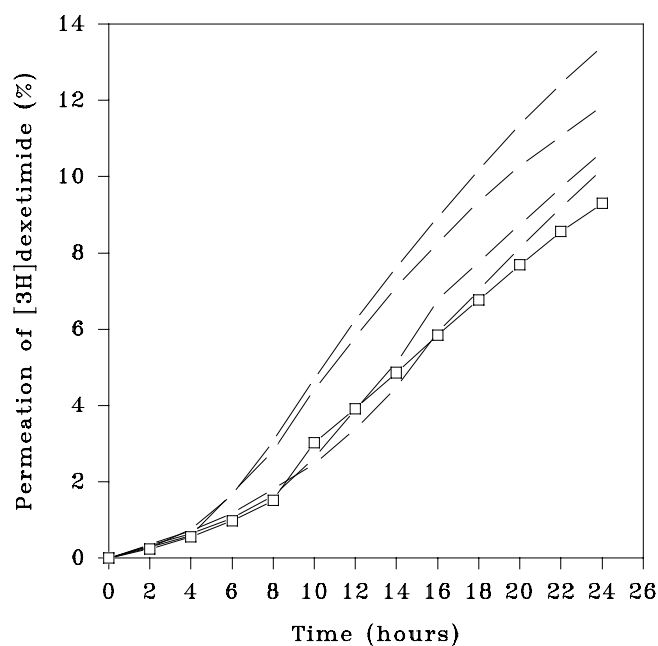


Figure 6.4. Effect of temperature on the permeation of [^3H]dexetimide through Silastic® membranes.

▼ = 22°C ($n=3$); ♦ = 27°C ($n=4$); □ = 32°C ($n=5$); ▲ = 37°C ($n=4$); ● = 42°C ($n=5$).

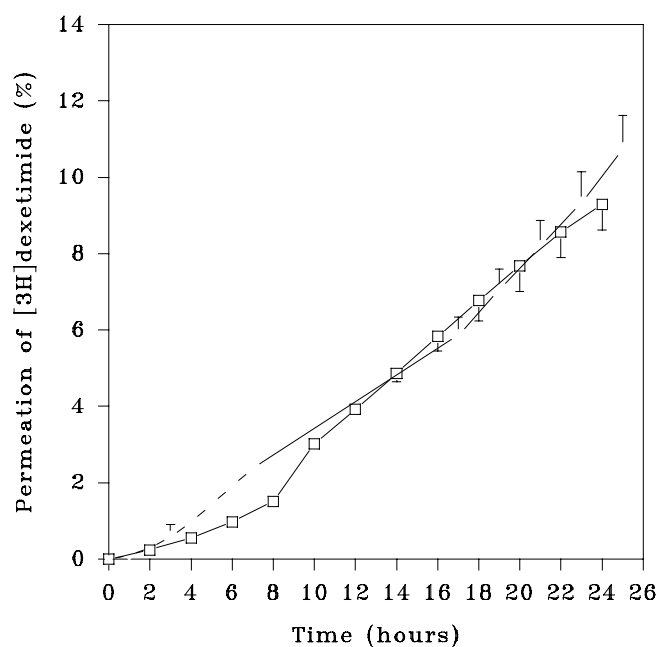


Figure 6.5. Permeation of [^3H]dexetimide through Silastic® membranes using static Franz diffusion cells and Kelder-cells.

□ = Kelder-cell ($n=5$); ▼ = Franz diffusion cell ($n=5$); each point represents the mean and standard error of the mean.

Table 6.1. Permeation of [^3H]dextimide at different temperatures : Means and standard error of the means.

Sampling time (hours)	22°C (n=3)	27°C (n=4)	32°C (n=5)	37°C (n=4)	42°C (n=5)
2	0.33 (0.01)	0.35 (0.05)	0.25 (0.01)	0.30 (0.06)	0.28 (0.02)
4	0.65 (0.01)	0.73 (0.06)	0.56 (0.02)	0.59 (0.12)	0.75 (0.04)
6	1.04 (0.01)	1.17 (0.10)	1.00 (0.04)	1.70 (0.39)	1.65 (0.07)
8	1.62 (0.08)	1.80 (0.06)	1.51 (0.08)	2.80 (0.67)	3.06 (0.10)
10	2.63 (0.23)	2.47 (0.12)	3.02 (0.09)	4.42 (0.89)	4.70 (0.10)
12	3.89 (0.33)	3.39 (0.16)	3.92 (0.08)	5.80 (1.06)	6.24 (0.12)
14	5.13 (0.36)	4.48 (0.17)	4.86 (0.22)	7.12 (1.14)	7.63 (0.20)
16	6.76 (0.34)	5.92 (0.35)	5.84 (0.38)	8.24 (1.22)	8.92 (0.31)
18	7.77 (0.31)	7.02 (0.30)	6.77 (0.54)	9.35 (1.31)	10.18 (0.38)
20	8.74 (0.27)	8.14 (0.26)	7.68 (0.67)	10.28 (1.38)	11.37 (0.42)
22	9.71 (0.28)	9.19 (0.26)	8.56 (0.67)	11.06 (1.46)	12.44 (0.47)
24	10.64 (0.31)	10.20 (0.29)	9.30 (0.68)	11.84 (1.52)	13.40 (0.51)

Table 6.2. Variability in [^3H]dextimide permeation through Silastic® membranes using Franz diffusion cells (n=6) and Kelder-cells (n=5).

Sampling time (hours) ^{*2}	Coefficient of variation ^{*1}	
	Kelder-cells	Franz diffusion cells
1	-	38.5
2	9.2	22.9
3	-	13.7
4	8.0	12.3
5	-	7.9
6	9.4	10.0
7/8	12.4	8.8
10	7.0	-
12	4.3	-
14	9.9	-
16/17	14.7	19.9
18/19	17.7	19.0
20/21	19.4	20.5
22/23	17.4	21.4
24/25	16.4	20.0

*1 Coefficient of variation = sd / mean * 100%.

*2 Sampling time is every two hours for the Kelder-cells; other time intervals are sampling times for Franz diffusion cells.

Thus, the two systems are capable of producing equivalent data, yet the Kelder-cell presents practical advantages over the Franz diffusion cell:

- The automatic sampling offers collection of two hours fractions which means a more accurate fitting of the permeation profile;
- Sink conditions are easily maintained by replacement of the buffer every two minutes, so saturation of the receptor fluid does not occur if the drug is being absorbed quickly or has limited water solubility;
- The Kelder-cells allow unattended operation for at least 24 hours.

6.4. Conclusions

The injection height of the needle into the inlet compartment of the cell should be programmed at -34 mm to ensure complete air tightness and force the buffer to flow through the cell. The volume of buffer pumped through the cell is important to completely remove the permeated chemical: A volume of 117 μ l was chosen to replace the total content of the cell (84 μ l) by fresh buffer every two minutes. The temperature of the entire system was precisely controlled in a thermostatic cabinet. Equivalent data were produced using Kelder-cells and Franz diffusion cells.

6.5. References

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